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Histology and immunohistochemistry of the human carotid sinus nerve

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Abstract: The carotid sinus nerve (CSN) is well known as mediating baroreflexes. However, studies of its detailed histological analysis are scant in the literature. Therefore, the current anatomical study sought to better elucidate the microanatomy of the CSN. Ten fresh frozen adult cadavers underwent dissection of the CSN. Then, it was harvested and submitted for histological and immunohistochemical staining. Specimens were all shown to be nerve fibers on histology and immunohistochemistry. We identified tyrosine hydroxylase positive fibers in all CSN specimens. These fibers were always found to be within the CSN and not on its surface *i.e.,* epineurium. Based on our findings, the majority of fibers contained in the CSN are tyrosine positive in nature. Further studies are necessary to understand the true function of this autonomic nerve fibers.

Key words: Carotid sinus nerve, Histology, Immunohistochemistry, Nerve stimulation, Anatomy

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Introduction

The carotid sinus nerve (CSN) originates from the glossopharyngeal nerve and carries afferent fibers from baroreceptors in the carotid sinus to the nucleus tractus solitarius in the brain stem [1, 2] as well as chemoreceptors from the carotid body for regulation of the respiratory rate [2, 3]. The nerve leaves the glossopharyngeal nerve deep to the combined posterior belly of the digastric and stylohyoid muscles and overlying hypoglossal nerve to then descend inferiorly along the anterior surface of the internal carotid artery (ICA), innervating the carotid sinus and carotid body (Fig. 1) [3]. The nerve, which has been reported to sometimes arise from the vagus nerve, is anterior to the vagus nerve and sympathetic trunk. The CSN contributes to the intercarotid plexus and is the major innervation of the carotid sinus [4].

The function of this nerve is to help maintain homeostasis through reflex mechanisms so that the ICA wall pressure is balanced via CSN activation within the carotid sinus [5]. Due to the many variants and communications with other surrounding nerves, including the vagus nerve and sympathetic trunk, the CSN has numerous alternative names including Hering's nerve, ramus descensus glossopharyngei (of Braeucker), and the intercarotid nerve (of De Castro) [3].

The CSN is well known as mediating the baroreflex that occurs at the carotid sinus. Traditionally, the reflex has been

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considered to have sensory input carried by the CSN and then the motor end of the reflex to be carried in the vagus nerve. Such a reflex, when stimulated, thus, reduces the heart rate/blood pressure. The ganglion where these sensory cell bodies are located is the inferior ganglion of the glossopharyngeal nerve. Additionally, the nerve has been suggested as a stimulation pathway for treating patients with medically intractable hypertension and epilepsy [6, 7]. However, studies of its detailed histological analysis are scant in the literature. Therefore, the current anatomical study sought to better elucidate the microanatomy of the CSN via histological analysis.

Materials and Methods

In the supine position, 10 fresh frozen adult cadavers (20 sides) with a mean age at death of 70.4 years (range 43–102 years) underwent dissection of the neck to identify the CSN. Five specimens were male and five were female. All specimens were less than five days postmortem. Dissection was carried out with forceps and scissors between the sternocleidomastoid muscle laterally and larynx/suprahyoid region medially. Loupe magnification was used during all dissections. The carotid sheath was identified and opened. Once the carotid bifurcation was verified, the origin of the ICA and carotid sinus was found. Next, the glossopharyngeal nerve was dissected deep to the styloid muscles and traced medially. The CSN branch of the glossopharyngeal nerve was localized arising from the glossopharyngeal nerve and traced inferiorly toward the carotid sinus. The CSN and its origin and course were documented with photography. Lastly, the CSN was harvested and placed in 10% formalin. Histological (cresyl violet-Luxol fast blue) and immunohistochemical stains (neurofilament, tyrosine hydroxylase) were then performed of the nerve. Paraffin-embedded specimens of the CSN were prepared and cut with 5 μm slices. Tyrosine

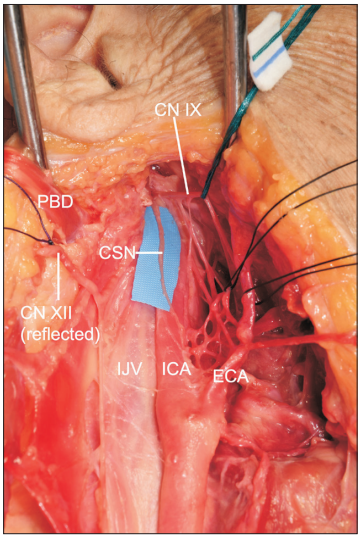

Fig. 2. Right cadaveric dissection noting the CSN. Note its origin from the CN IX. Also, observe the posterior belly of the digastric muscle (reflected laterally), the hypoglossal nerve (reflected laterally), the IJV, ICA, and ECA (cadaveric dissection from Tulane University). CSN, carotid sinus nerve; CN IX, glossopharyngeal nerve; IJV, internal jugular vein; ICA, internal carotid artery; ECA, external carotid artery; PBD, posterior belly of the digastric muscle; CN XII, hypoglossal nerve.

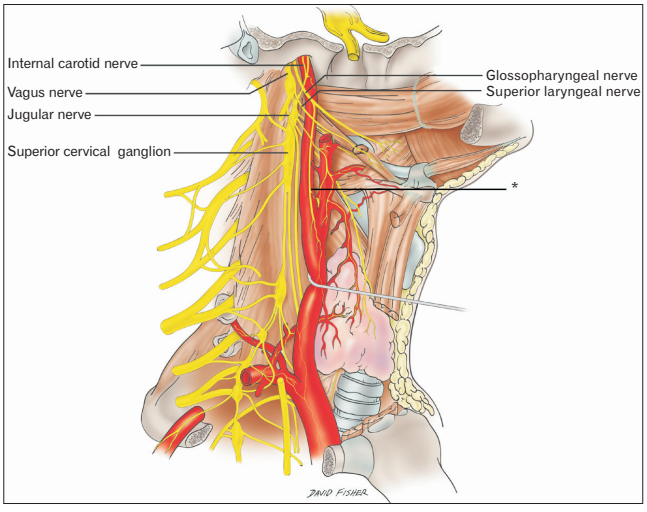

Fig. 1. Schematic drawing of the right side of the neck noting the major nerves. *The carotid sinus nerve. By David Fisher.

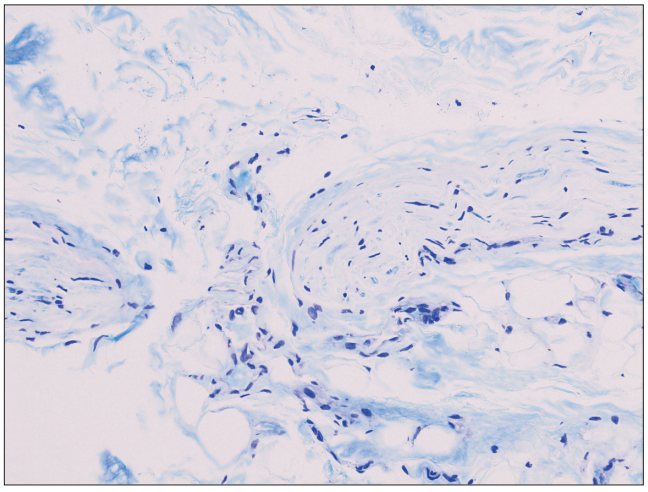

Fig. 3. Histological imaging (cross section) of the carotid sinus nerve (center of image) (×200; cresyl violet-Luxol fast blue).

hydroxylase (Vision BioSystems) was used to elucidate the sympathetic nature of the CSN. Neurofilament (Vision BioSystems) was used to verify nerve fibers in the specimens. The concentration of antibodies was 1:80. Antigen retrieval was conducted using Steamer-Tris-ethylenediamine tetraacetic acid (EDTA; Carpinteria) at a pH of 9.0 all prediluted with heat-induced epitope retrieval-Tris-EDTA at 98°C at a pH of 9.0 for 20 minutes and a cool down time of 20 minutes. Detection reagents included dual endogenous enzyme block S2003, Target Retrieval S1700, Envision Dual Link, LSAB 2 System HRP, DAB+K34568, and hematoxylin counterstain all from DakoCytomation. Standard controls for all of the aforementioned stains were used.

All specimens were harvested and studied at Tulane University. Tulane University Insitutional Review Board does not require approval of non-patient/living human studies. Thus, as our study used cadavers, approval was not required. No previous surgical scars or gross pathology was noted in the region dissected to expose the CSN. The authors state that every effort was made to follow all local and international ethical guidelines and laws that pertain to the use of human cadaveric donors in anatomical research [8].

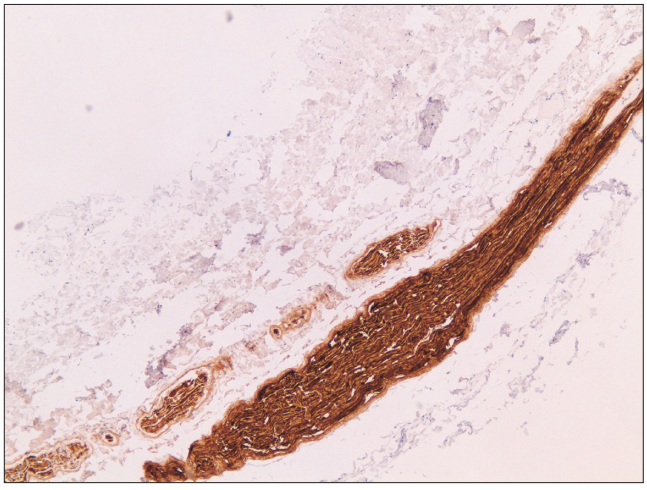

Fig. 4. Longitudinal section of the carotid sinus nerve (neurofilament ×400).

Results

A CSN was identified on all sides (Fig. 2). On two sides (one left and one right), the nerve was duplicated with both branches arising directly from the glossopharyngeal nerve. These were all shown to be nerve fibers on histology (Fig. 3) and immunohistochemistry (Fig. 4). We identified tyrosine hydroxylase positive fibers in the CSN confirmed with immunohistochemistry (Fig. 5). These fibers were always found to be within the CSN and not on its surface *i.e.,* epineurium. Approxiamtely 75% of each CSN was found to be tyrosine positive in our study. No signs of trauma or previous surgery were identified in the regions dissected of any specimen. Other than the two sides found to have duplicated CSNs, no other additional anatomical variations in the areas dissected were identified.

Discussion

Tyrosine hydroxylase positive reactivity was found in all CSN in our study. These fibers were within the CSN suggesting they are intrinsic parts of this nerve and not fibers traveling along its outer nerve sheath. Although there are connections with the lower cranial nerves and the sympathetic trunk/superior cervical ganglion at the skull base, past studies have not found a preponderance of tyrosine positive

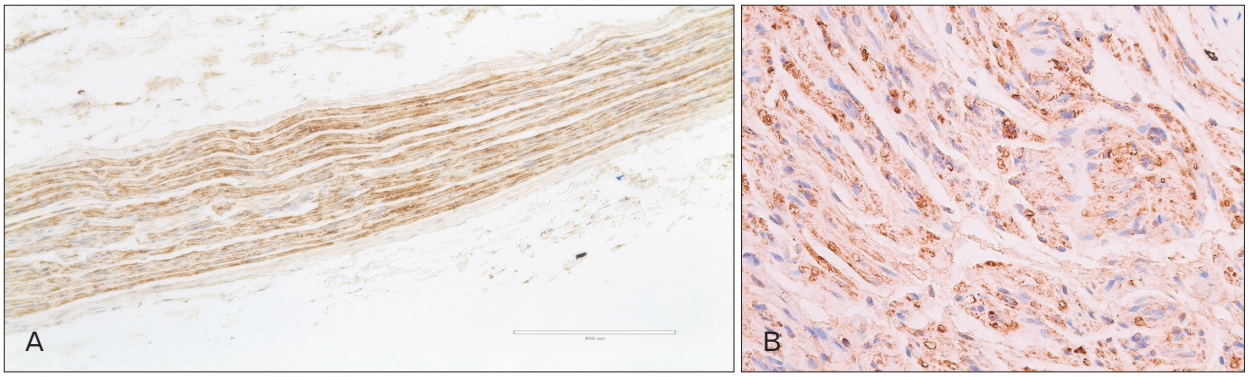

Fig. 5. (A) Longitudinal (×40) (B) and axial sections (×400) of the carotid sinus nerve both stained with tyrosine hydroxylase.

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fibers intrinsically within the CSN [9]. The CSN branches most commonly directly off the glossopharyngeal nerve following its exit from the jugular foramen and courses to supply the carotid sinus and carotid body [3]. The CSN has been demonstrated to contain afferent fibers from baroreceptors and chemoreceptors to control blood pressure [3]. Histologically, the CSN terminates in the carotid sinus, which has a thick tunica adventitia and a thin tunica media. These features allow the blood pressure, which interacts with elastin at the medio-adventitial border of the carotid sinus and with collagen inside the adventitia [10], to be sensed readily by the nerve endings [11]. Both collagen and elastin are fibrous connective tissue components that provide strength and flexibility to structures. Arteries contain an abundance of elastin in their tunica media that allows their walls to stretch in response to pulsating blood, and the abundance of collagen in their tunica adventitia is used for support and to prevent overexpansion of the vessel [10].

The CSN has both type I and II baroreceptors. Type I receptors are larger A-fibers within the rostral dorsomedial subnucleus, while type II are smaller and are located within the commissural, medial, and dorsal subnuclei of the nucleus tractus solitarius [12]. The difference in myelination between these fiber types is reflected in the speed with which they convey sensory information. The type I receptors have faster conduction velocities; the type II receptors are slower. The myelinated type I receptors communicate fluctuations in arterial blood pressure to the brainstem so that cardiovascular neurons can attenuate flow to the heart and peripheral arteries [13]. The type II receptors can be classified as smaller A-fibers and unmyelinated C-fibers, both of which regulate tonic blood pressure control [5].

Electron microscopy reveals these nerve fibers in the adventitia of the carotid sinus. Blood pressure regulation is crucial as it not only prevents surges in arterial pressure that can be lethal but also these baroreceptor neurons detect pulsatile and acute fluctuations that allow for improved blood pressure control and protect homeostasis [14]. With regard to hypoxia, stimulation of the carotid body in rats by hypoxia has identified tyrosine hydroxylase positive neurons in the petrosal ganglion [15]. As we found tyrosine hydroxylase positive reactivity in the CSN itself, this suggests that the CSN may be directly involved in the efferent processes as well. However, the source of these fibers is not fully understood. Similarly, Yokoyama et al. [16] described in rats that the vesicular glutamate transporter 2 is localized in the afferent nerve terminals of the carotid body which were immunoreactive to P2X3, therefore, glutamate may be released from these terminals and control the chemosensory activity of the caroty body, particularly during hypoxia-evoked activity of the CSN.

Connections between the CSN and adjacent cervical sympathetic fibers has been scantly mentioned in reports in the literature however would have significant applications to treatment for carotid sinus hypersensitivity and carotid sinus syndrome [17]. While it is known that sympathetic nerve fibers from the superior cervical ganglion may reach the CSN [5], our findings reveal tyrosine positive fibers within the CSN itself, rather than only traveling to the carotid sinus via the external plexus. Interestingly, in Hering's initial studies, he found with transection of the CSN, the "heart's inhibitory and vasodilatory" reflexes are lost, leading to loss in rise in blood pressure and heart rate when the common carotid artery was occluded [2]. Although our study identified that much of the axons within the CSN are sympathetic in nature, the true action of these fibers is not known. However, future animal studies of this physiology might add to our knowledge of why autonomic fibers are found in the CSN.

Early work investigating the role of the carotid body and CSN on hypertension revealed that bilateral destruction of the CSN reduced the blood pressure in hypertensive rats and reduced sympathetic vasomotor tone, preventing future hypertension [18-20]. In a rat model, Conde [19] and Conde et al. [20] demonstrated that blocking the CSN led to a global loss of sympathetic tone. In addition, the increase in cardiac sympathetic activity in response to hypoxia was limited [19, 20]. Given the proximity of the chemoreceptor and baroreceptor reflexes, the authors highlight the difficulty in selectively targeting the CSN [19, 20]. In addition, the authors explain that the diminished expected effects of CSN ablation could be explained by another compensatory mechanism outside of the carotid body and CSN that help preserve autonomic function [19, 20].

The manipulation of the CSN and individual receptors within the carotid body during procedures such as a carotid endarterectomy may be difficult and cause injuries. This and other types of damages involving the CSN due to neck trauma and radiation therapy have direct effects on blood pressure and heart rate control, requiring further managments to mediate these injuries and the pathologic processes already mentioned. These effects on blood pressure fluctuation are caused by damage to the unmyelinated nerves of the CSN that are embedded in the wall of the carotid artery and

are highly sensitive to manipulation and or direct trauma to the muscle surrounding the carotid [3].

Disease of the CSN can be life threatening, especially in the elderly, as carotid sinus pathology may lead to syncopal events and falls, often secondary to changes in blood pressure and heart rate [3]. Carotid sinus syndrome, of which there are three classifications, develops in elderly patients over the age of 75 years and can result in asystole, significant hypotension, and amnesia with the best treatment option currently being carotid massage, although, denervation of the carotid sinus has also been proposed to treat the carotid sinus syndrome [3, 5]. Carotid sinus syndrome is difficult to diagnose as its clinical picture is similar to that of vasovagal or idiopathic syncope and polypharmacy can cloud the diagnosis [3]. In older patient populations or in anyone on a blood thinner, these events can result in orthopedic injuries, intracranial hemorrhage, and covert intraabdominal injuries, all of which can prove lethal, especially in patients with multiple medical comorbidities [3]. Carotid sinus stimulation has proven difficult and with variable effects given the wide variety of patient symptomatology and often mixed pathology [17]. Highlighting the complexity of this anatomical region, while prior carotid sinus stimulation was successful for a subgroup of patients with angina given the modulation on blood pressure and resulting decrease in blood pressure, several patients in the landmark studied passed from complications related to hypoxia and hypotension intraoperatively [21]. More recent work with modern carotid sinus stimulators revealed similar improvement in control of hypertension [22].

This study is not without limitations. These include a population that is exclusively adult in nature. Additionally, only 20 sides were dissected. In so much, additional specimens might have shown greater variation. Lastly, the population was from a single geographical area. Therefore, a cohort from a wider ethnic background might shed more light on this nerve's anatomy and histology.

Based on our findings, the majority (75%) of fibers contained in the CSN are tyrosine hydroxylase positive. These data might explain unusual complications following dissection near or stimulation of this nerve. Further studies are necessary to understand the true function of these autonomic nerve fibers.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. Toorop RJ, Scheltinga MR, Moll FL, Bleys RL. Anatomy of the carotid sinus nerve and surgical implications in carotid sinus syndrome. J Vasc Surg 2009;50:177-82.
2. Shoja MM, Rai R, Lachkar S, Iboroma Akobo S, Yilmaz E, Loukas M, Binello E, Gorjaian M, Griessenauer CJ, Iwanaga J,

https://doi.org/10.5115/acb.23.084

Tubbs RS. The carotid sinus nerve and the first English translation of Hering's original research on this nerve. Cureus 2019;11:e3898.
3. Kikuta S, Iwanaga J, Kusukawa J, Tubbs RS. Carotid sinus nerve: a comprehensive review of its anatomy, variations, pathology, and clinical applications. World Neurosurg 2019;127:370-4.
4. Andrew K, Iwanaga J, Loukas M, Oskouian RJ, Tubbs RS. A variant origin of the carotid sinus nerve. Cureus 2018;10:e2883.
5. Porzionato A, Macchi V, Stecco C, De Caro R. The carotid sinus nerve-structure, function, and clinical implications. Anat Rec (Hoboken) 2019;302:575-87.
6. Tubbs RS, Wellons JC 3rd, Patwardhan RV, Oakes WJ, Wyss JM. The effectiveness of Hering's nerve stimulation in controlling penicillin-induced seizures in the rat is dependent on the amygdala. Pediatr Neurosurg 2002;37:231-4.
7. Tubbs RS, Patwardhan RV, Wellons JC 3rd, Oakes WJ. Cortical representation of Hering's nerve: a possible anatomical pathway for seizure cessation following electrical stimulation. Pediatr Neurosurg 2002;37:235-9.
8. Iwanaga J, Singh V, Takeda S, Ogeng'o J, Kim HJ, Moryś J, Ravi KS, Ribatti D, Trainor PA, Sañudo JR, Apaydin N, Sharma A, Smith HF, Walocha JA, Hegazy AMS, Duparc F, Paulsen F, Del Sol M, Adds P, Louryan S, Fazan VPS, Boddeti RK, Tubbs RS. Standardized statement for the ethical use of human cadaveric tissues in anatomy research papers: recommendations from Anatomical Journal Editors-in-Chief. Clin Anat 2022;35:526-8.
9. Kawagishi K, Fukushima N, Yokouchi K, Sumitomo N, Kakegawa A, Moriizumi T. Tyrosine hydroxylase-immunoreactive fibers in the human vagus nerve. J Clin Neurosci 2008;15:1023-6.
10. Rees PM. Observations on the fine structure and distribution of presumptive baroreceptor nerves at the carotid sinus. J Comp Neurol 1967;131:517-48.
11. Boyd JD. Observations on the human carotid sinus and its nerve supply. Anat Anz 1937;84:386-99.
12. Dean C, Seagard JL. Mapping of carotid baroreceptor subtype projections to the nucleus tractus solitarius using c-fos immunohistochemistry. Brain Res 1997;758:201-8.
13. Seagard JL, van Brederode JF, Dean C, Hopp FA, Gallenberg LA, Kampine JP. Firing characteristics of single-fiber carotid sinus baroreceptors. Circ Res 1990;66:1499-509.
14. Drummond HA, Price MP, Welsh MJ, Abboud FM. A molecular component of the arterial baroreceptor mechanotransducer. Neuron 1998;21:1435-41.
15. Finley JC, Polak J, Katz DM. Transmitter diversity in carotid body afferent neurons: dopaminergic and peptidergic phenotypes. Neuroscience 1992;51:973-87.
16. Yokoyama T, Nakamuta N, Kusakabe T, Yamamoto Y. Vesicular glutamate transporter 2-immunoreactive afferent nerve terminals in the carotid body of the rat. Cell Tissue Res 2014;358:271-5.
17. Parry SW. Should we ever pace for carotid sinus syndrome? Front Cardiovasc Med 2020;7:44.
18. Abdala AP, McBryde FD, Marina N, Hendy EB, Engelman ZJ, Fudim M, Sobotka PA, Gourine AV, Paton JF. Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat. J Physiol 2012;590:4269-77.
19. Conde SV. Ablation of the carotid bodies in disease: meeting its adverse effects. J Physiol 2018;596:2955.
20. Conde SV, Sacramento JF, Melo BF, Fonseca-Pinto R, Romero-Ortega MI, Guarino MP. Blood pressure regulation by the carotid sinus nerve: clinical implications for carotid body neuromodulation. Front Neurosci 2022;15:725751.
21. Epstein SE, Beiser GD, Goldstein RE, Redwood D, Rosing DR, Glick G, Wechsler AS, Stampfer M, Cohen LS, Reis RL, Braunwald NS, Braunwald E. Treatment of angina pectoris by electrical stimulation of the carotid-sinus nerves. N Engl J Med 1969;280:971-8.
22. Bisognano JD, Bakris G, Nadim MK, Sanchez L, Kroon AA, Schafer J, de Leeuw PW, Sica DA. Baroreflex activation therapy lowers blood pressure in patients with resistant hypertension: results from the double-blind, randomized, placebo-controlled rheos pivotal trial. J Am Coll Cardiol 2011;58:765-73.
23. Iwanaga J, Singh V, Ohtsuka A, Hwang Y, Kim HJ, Moryś J, Ravi KS, Ribatti D, Trainor PA, Sañudo JR, Apaydin N, Şengül G, Albertine KH, Walocha JA, Loukas M, Duparc F, Paulsen F, Del Sol M, Adds P, Hegazy A, Tubbs RS. Acknowledging the use of human cadaveric tissues in research papers: recommendations from anatomical journal editors. Clin Anat 2021;34:2-4.